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Original Article

Biological Activity of Ethanol Extract of *Terminalia chebula* Dried Carp against Bacterial Wilt of *Lycopersicum esculentum* and its Mechanism of Inhibition

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ABSTRACT

Background: The extract of *Terminalia chebula* was explored with a new perspective to control tomato bacterial wilt, opening up a new way in agriculture to control plant disease in an organic way with less impact on the environment, ecosystem and consumers. **Materials and Methods:** The preliminary study reveals the presence of phenolic compounds, coumarins and anthroquinones in the ethanol extract and was analysed further by GCMS and NMR to identify the compounds, structure and functional group in the EE. The macro-dilution superior to diffusion method to find the rate of inhibition to measure the potential of phytochemicals in Ethanol Extract (EE) **Results:** The optimum of 0.1 mg/mL of crude EE inhibits 56% of *Ralstonia solanacearum* (RS) effectively up to 96% with 0.8 mg/mL. The extent of the *in vitro, in vivo* experiment on tomato (*Lycopersicum esculentum*) to check any toxic compounds in EE that hinder plant growth and the absence of such compounds makes the EE a good source of organic alternatives for synthetic compounds. Simultaneously to find biologically active compounds reduces the disease severity in the inoculated plant. The pot culture experiments were conducted with seeds and plants of *Lycopersicum esculentum* that received EE against induced RS infection. **Conclusion:** The five phytochemicals in crude ethanol extract have bioactivity on host plants inoculated with *Ralstonia solanacearum*, the severity of the disease decreased with that of the diseased plant not receiving any treatment. The extract-treated plant was measured in height and weight to non-treated control plants and there was a significant difference between groups. Identified compound affinity was measured with molecular docking and interpreted.

Keywords: *Ralstonia solanacearum*, *Terminalia chebula*, Antibacterial activity, Macro-dilution method, Pot culture, Organic control.

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INTRODUCTION

Milky secretion observed with a cut along with wilting of green leaves and drooping of leaves is the major indication of Bacterial Wilt (BW) of tomato plants observed in Tamil Nadu a southern state of India where 30-45% of plants infected. Pathogen *Ralstonia solanacearum* upon inoculation in a potted tomato plant produced BW within 4-5 days. Genetic analysis indicates the pathogen clusters with outbreaks in Andaman and Nicobar Island and Kerala indicate that the pathogen spreads too many parts of India with the expansion of tomato cultivation.[1]

R. solanacearum gains entry through contaminated seeds to cotyledons *Lycopersicum esculentum* (tomato).[2] Pathogen wilts its host within 1 to 3 weeks[3] after inoculation warm and tropical weather makes pathogens thrive well in tomatoes than in any other crops. It has a wide range of hosts including 450 plant species with widespread weather in tropical, subtropical and temperate regions[4] suits the pathogen growth. Gain enters to tomato and grows in xylem vessels clogging to death.[5]

BW or brown rot of potato incident occurred in mid hills of Meghalaya in India.[6] There were many reports from India of BW in eggplant and chilli of Solanacearum crops[7] making this pathogen widespread in the mainland of India as well as on the Islands associated with India. From 2005 to 2010 reports from Bengal confirms the wide spread of BW in many crop plants[8] wide host range and well adaptation to climate makes its survival potential very high. Bacterial Wilt (BW) caused by *R. solanacearum* of crop one of the most devastating diseases in the world leads to huge economic loss and a reduction in the yield.

In recent years researchers focus on the green way of controlling the BW with bactericidal compounds, soil disinfection, resistant

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plant variety, usage of antimicrobial plant extract, agrochemicals, genetic modification, biocontrol and bacterial antagonistic gaining importance because of less impact on the environment attract the scientists and researchers. One such is using rhizosphere competency with inoculation of biocontrol agent to suppress *Ralstonia solanacearum* (Elsayed, T.R., *et al.*, 2020, Anuratha, C.S. *et al.,* 1990) some strains were promising but with change in rhizosphere microbiota. Eco-friendly control measures (Singh, S., *et al.*, 2014) like grafting technique and cocopeat added to growing plants give protection range from 5% to 45%. Phytobiocids are good alternatives for BW many herbal extracts tested has a wide range of control[9-11] promising alternatives for synthetic bactericidal compounds.

This BW is not only confined to India but to the entire world from small to vast tomato producers in the entire world. As a developing country taking down this disease by economically affordable to the farmers unites the researchers and the scientists to overcome this disease and produce valuable yield from the crops.

The primary objective of this study is to control one of the most serious plant pathogens with medicinal plant *T. chebula* extract without affecting the environment, ecosystem and the consumer. The king of medicine *T. chebula* very well-known plant with different applications makes added value to it. Under pot culture, the pathogen was inoculated and compared with the infected tomato treated with *T. chebula* extract efficacy of the extract on the infected plant was studied and compared. There are many synthetic compounds used to control this disease but prolonging usage affects the environment and evolution of resistance in the pathogen, changes the ecological chain and causes health ailments to those who consume.

MATERIALS AND METHODS

Collection, processing and Extraction of sample

The dried fruits (*T. chebula*) were collected from Gudalur district in Tamil Nadu during February, visually inspected and appropriate fruits were selected and authenticated. The outer epicarp was separated from seeds and was used in this study. The extracts of the plant materials were obtained using the cold extraction method.[12] 100 g of powdered *T. chebula* fruit materials (epicarp) were weighed into sterile conical flasks with 500 mL of ethanol left for 48 hr at room temperature. The resultant suspensions were filtered into sterile conical flasks. These extracts were air-dried and the residue was collected, they have been labelled appropriately and used for further studies.

Preliminary detection of phytochemicals

The analysis was made for the presence of sugars, amino acids, anthroquinins, coumarins, saponins, tannins, phenolic compounds, flavonoids, terpenoids and glycosides[13] in crude ethanol extract of dried epicarp of *Terminalia chebula*.

GCMS analysis

Epi part ethanol extract of *T. chebula* dried fruit analysed by GCMS (GC/MS -series QP2010, Shimadzu, Tokyo, Japan) using Thermal Desorption (TD) system. The GC-MS had an Rtx- capillary column (DB 35-30 m x 0.25 mm x 0.25 μm). Pure helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1.21 mL/min. For GC-MS spectral detection, an electron ionization energy method was adopted with high ionization energy of 70 eV (electron Volts) with 0.2 s of scan time and fragments ranging from 50 to 650 m/z. The injection quantity of 1 μL was used (split ratio 10:1) and the injector temperature was maintained at 250ºC (constant). The column oven temperature was set at 50ºC for 3 min, raised at 10ºC per min up to 280ºC and the final temperature was increased to 300ºC for 10 min. The contents of phytochemicals present in the test samples were identified based on a comparison of their retention time (min), peak area, peak height and mass spectral patterns with that spectral database of authentic compounds stored in the National Institute of Standards and Technology (NIST) library.

NMR

^{1}H NMR

^{1}H spectra were recorded on a Bruker 400 MHz NMR Spectrophotometer. The proton pulse width was 12.25 s. A sample concentration of about 40 mg of the sample dissolved in DMSO-d6 (Dimethyl Sulfoxide-d6) was used for recording the spectra at 35ºC. About 100-200 scans were accumulated to get a good spectrum. The region between 0-10 ppm was recorded for all the samples. Chemical shift values were expressed in ppm relative to internal Tetramethylsilane (TMS) as the standard.

^{13}C NMR

^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. A sample concentration of about 40 mg dissolved in DMSO-d6 (Dimethyl Sulfoxide-d6) was used for recording the spectra at 35ºC. About 500 to 2000 scans were accumulated for each spectrum in the 0-200 ppm region.

Biological properties

Antioxidant assays

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay

ABTS radical scavenging assay of epicarp was performed according to the modified method of Perumal.[14] The ABTS (7 mM, 25 mL in deionized water) stock solution was prepared with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 μL). Different concentrations of test samples and standard (Ascorbic acid) were mixed with the ABTS working solution (2.0 mL) and the mixture was allowed to stand at room temperature for 20 min; then, the Absorbance was measured using an ultraviolet-visible

spectrophotometer at 734 nm. The ABTS radical scavenging effect was calculated by the equation:

ABTS radical scavenging effect (%)=[(A0-A1)/A0]×100, respectively.

Where,

A0 is the control (ABTS radicle+solvent).

A1 is the test (ABTS radical+solvent extract).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

DPPH radical scavenging assay of samples (ENDO) and (EPI) was performed according to the modified method described by Perumal,[14] 2018. In brief, 0.135 mM DPPH was prepared in methanol. Different concentration of extract (5, 10, 20, 40, 80, 160 and 320 µg/mL) was mixed with 2.5 mL of DPPH solution. The reaction mixture was vortexed thoroughly and kept at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference standard. The ability of plant extract to scavenge DPPH radical and control was calculated using the following formula.

% DPPH inhibition=[(OD of control-OD of test)/(OD of control)]×100

Macro-dilution method (*In vitro* test)

R. solanacearum BI 0001 was allowed to grow in a nutrient agar medium and incubated at 28ºC. A single colony was picked off and placed in a nutrient broth medium and incubated overnight on the rotary shaker at 180 rpm, 30ºC. To100 µL of bacterial suspension (1×108 CFU/mL), 100 µL of *T. chebula* (ethanol) extract of various concentrations ranging from 0.1, 0.2, 0.4, 0.6, 0.8 mg/mL were added separately and incubated at 30ºC on a rotary shaker at 180 rpm for 48 hr. While the same volume of sterile distilled water was used with the bacterial suspension as a control. Optical readings were taken at 600 nm at every 12 hr interval for up to 2 days. All procedures described above were carried out under sterile conditions and experiments were done in three replicates and the average was calculated. The mean inhibition of growth was calculated by (Ac-At)/Ac*100, Ac-Average of light absorption value and at a particular wavelength of Negative control, At- Average of light absorption value at a particular wavelength of the samples. The effectiveness of the sample compared with positive control Ampicillin.

Pot culture experiment (*in vivo* test)

In vivo experiment was carried out in a unique way of pot culture condition to study the effect of ethanol epi part extract of *T. chebula* dried fruit at three different concentrations on the tomato seeds for toxicity, the effect of crude extract on growing plants also during vegetation and the effect of crude extract on pathogen inoculated seedling for a potential candidate of disease control agent.

Seeds of tomato (*L. esculentum*) were obtained from Tamilnadu Agricultural University, Coimbatore and stored at 4ºC. The seeds were washed with sterile distilled water and placed in extract (0.05, 0.1 and 0.2% w/v) solutions (20 seeds/2 mL) separately and kept in a rocker at room temperature overnight. For control, seeds were placed in sterile water under the same condition. The seeds (1-2 seeds/pot) were then sown in polythene bags (21 cm in height, 16 cm in diameter) containing 2 kg of soil. The soil mixture contains red soil and farmyard manure (3:1) suitable to nourish the plant in a greenhouse. For foliar spray particular concentrations of extract 0.05, 0.1 and 0.2%, w/v were applied separately to 20th-day-old tomato plants and the next spray to the 35th-day-old plant.

The control and treated 21-day-old seedlings were thoroughly washed with sterile distilled water and predisposed to nearly 95% humidity for 8 hr. The suspension from 12 hr old *R. solanacearum* culture (1×108 CFU/mL), was inoculated by soil infestation on the control and treated tomato plants. The soil was infested by adding 1 mL of the bacterial cell suspension around the stem base of each plant. The visible symptom of the disease was monitored in tomato plants daily for the next 25 days.[15]

Statistical analysis

All statistical analyses were conducted using SPSS 21 software support and all data were analysed further with graph pad prism version 5.0. To deliberate the data descriptive statistics, Mean and SD were used. To find the significant difference between samples one-way ANOVA followed Duncan's test was performed. Based on the probability values <0.01 and 0.05 levels were considered as significant and highly significant respectively.

Docking

Docking studies reveal the mechanism of phytochemicals in crude ethanol extract of epi part of *T. chebula* dried fruit on plant pathogen *Ralstonia solanocearum*. The drug likeliness of phytochemicals (based on GCMS results) in crude ethanol extract alone obeys Lipinski's rule, only for those phytochemicals, molecular docking has been done. The Drug-likeness and properties of the compounds were evaluated by admetSAR and molinspiration webservers.[16,17] The drug likeliness was evaluated based on Lipinski's rule of five.[18]

Molecular docking studies were carried out using AutoDocVina[19] to calculate their binding affinity with the ligand. The targets are Dihydrolipoamide dehydrogenase (PDB ID: 4JDR) and Citrate synthase (PDB ID: 6ZU0). The grid boxes used for the proteins 4JDR are of the sizes 52 x 46 x 46 and 6ZU0 are sized 60 x 60 x 60. The docking region was determined by removing the pre-existing ligand, in the PDB file and re-docking it with the protein. The 3D

docked protein-ligand complex was imaged using PyMolecular software.[20]

RESULTS AND DISCUSSION

Detection of Phytochemical compounds

Preliminary analysis of phytochemical constituents reveals the absence of Flavonoids, Steroids, Sugars, Saponins, Terpenoids and Tannins in contrast to Choudhary, R.A., *et al.*, 2021[21] who confirmed the presence of sterols and terpenoids. A moderate amount of reducing sugars and aminoacids, a high amount of Phenolic compounds, Glycoside, Coumarins and Anthroquinones were present. To the extend GCMS revealed the presence of 20 phytochemicals in crude ethanol extract of *Terminalia chebula* dried fruits, 18 phytochemicals (Figure 1) were identified with their structure. EE good source of 9-Octadecenoic acid (67.22%) regard to the study conducted in 2017 by Ryu J *et al.*, 27.4% in the seeds of kenaf plant.[22] The second most abundant n-Hexadecanoic acid possesses some biological activity like antioxidant, nematicide and pesticide.[23]

The most abundant 9-Octadecenoic acid, (E) highest peak area (67.22) with anti-oxidant activity,[24] anti-bacterial[25] and have medicinal value.[26] The n-Hexadecanoic acid a second abundant compound, peak area (15.27) with anti-bacterial and

5-Hydroxymethylfurfural

Cis-13-Eicosenic acid

cis-9, cis-12-Octadecadienoic acid

n-Hexadecanoic acid

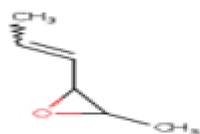
2,2-Bis(4'-methoxyphenyl)propane

n-Tridec-1-ene

Hexadecyl bromoacetate

trans-δ9-Octadecenoic acid

2-Methyl-3-prop-1-enyloxirane



3,5-Dimethyl-1-dimethylphenylsilyloxybenzene

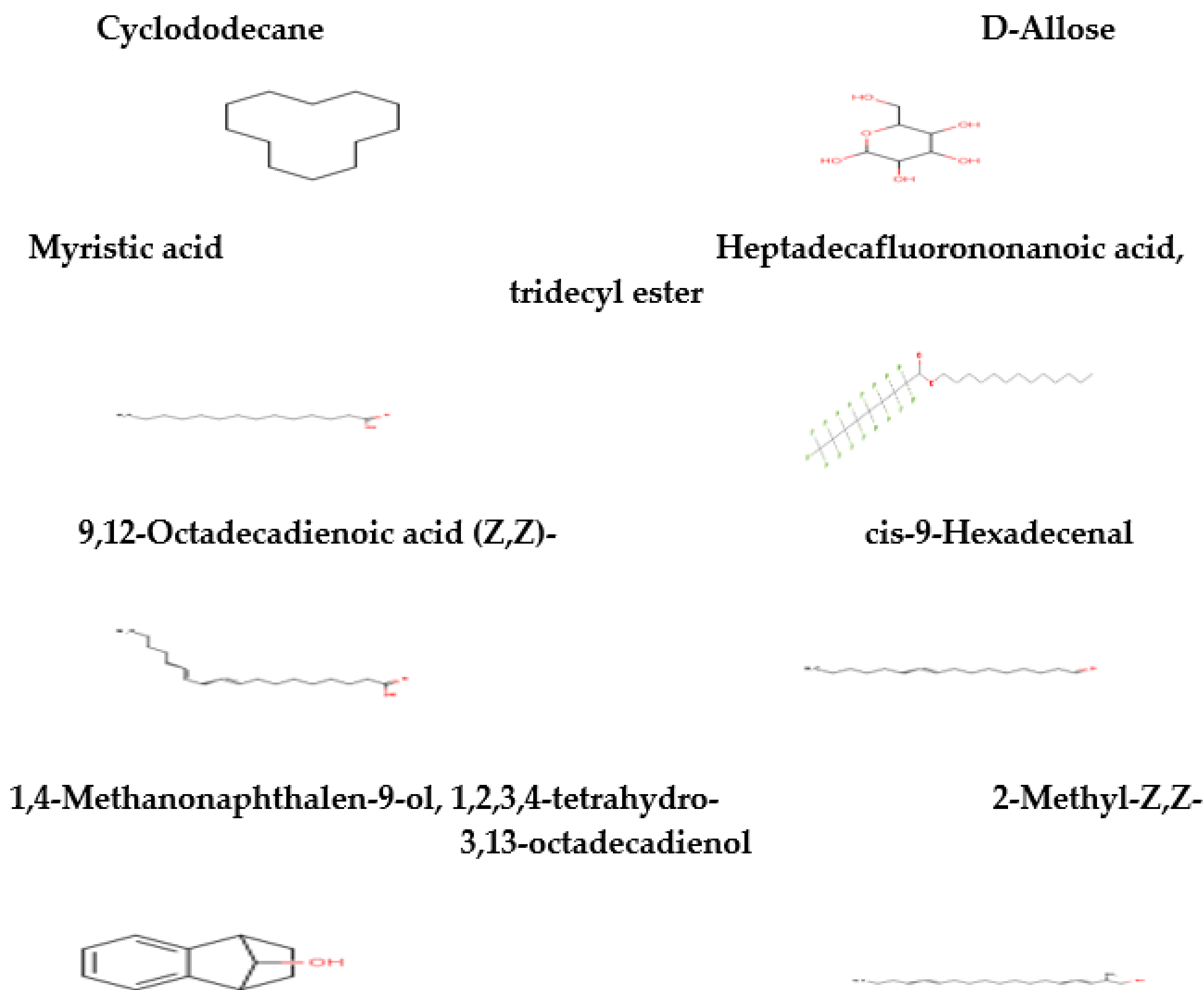


Figure 1: Structure of biologically active compounds in ethanol extract.

anti-fungal activity[27] in ethanol extract of epi part *T. chebula*. Other major compounds were 5-Hydroxymethylfurfural (confer anti-microbial activity by Makarewicz, M., *et al*., 2017,[28] D-Allose, 9,12-Octadecadienoic acid (Z,Z)- a subclass of lineolic acid and its derivative, cis-13-Eicosenoic acid and Zinc, bis[[5,5'-methylenebis[3,4-dihydro-4,4-dimethyl-2H-pyrrol-2-onato]](1-)-N1,N1']-, (T-4)- identified in EE. Likewise Gupta, D. and Kumar, M also isolated cis13-eicosenoic acid and cis vaccenic acid from the methanol extract of *Terminalia arjuna*.[29]

The H1 NMR (Figure 2) spectrum shows an aromatic ring as δ range 7.0-8.0 ppm and aliphatic chain and also has DMSO and water content. C13 NMR (Figure 3) also help to understand the carbon environment of the crude sample with C-C, C-O, C=C and C=O and co-inside with GCMS search. There were 7 peaks representing 7 different carbon. The first peak near 160 to 180 confirms C=O (as in acid and ester), second and third peaks between 125 to 150 C in aromatic rings, peak third and four C=C in alkene 115 to 140, peak five between 50-90 C in RCH_2O, peak 6 and 7 C in RCH_2NH_2 and RCH_2Cl either environment. There were C engaged with C-C, C-O, C=C and C=O containing compounds present in the ethanol extract of dried fruit. Further C13 NMR concludes component has 8 carbon molecules.

NMR

H1 NMR has an aromatic ring structure and aliphatic chain and also has DMSO and water content.

Antioxidant properties

The phytochemicals in the crude dried fruit ethanol extract exhibit better antioxidant activity (Table 1) with ABTS and DPPH with standard Vitamin C (Gul R, et al., 2017).

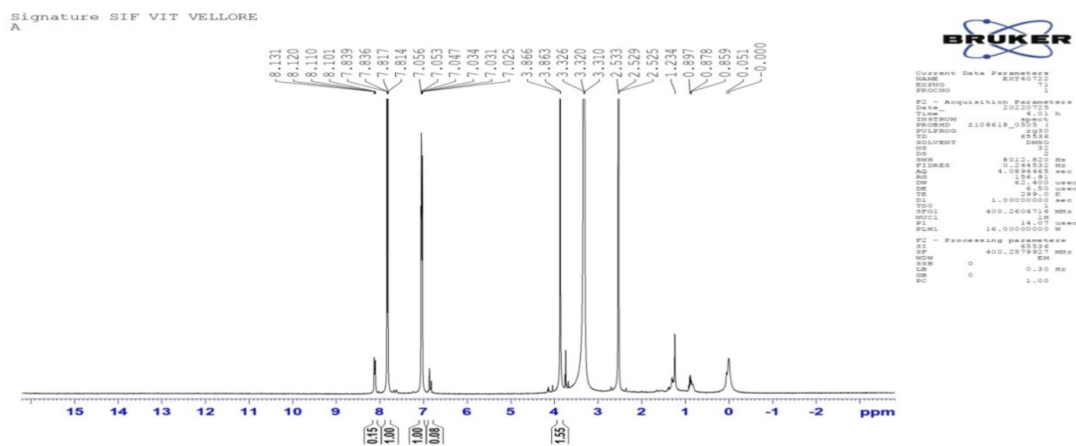


Figure 2: H^1 NMR.

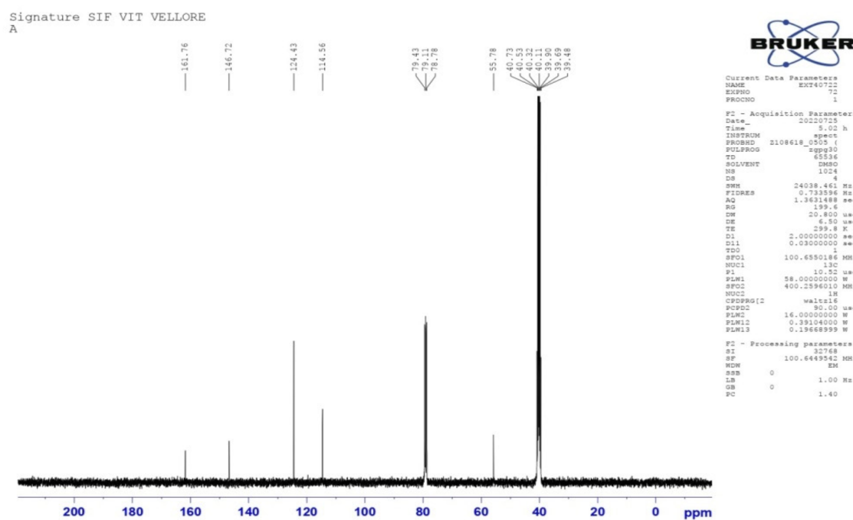
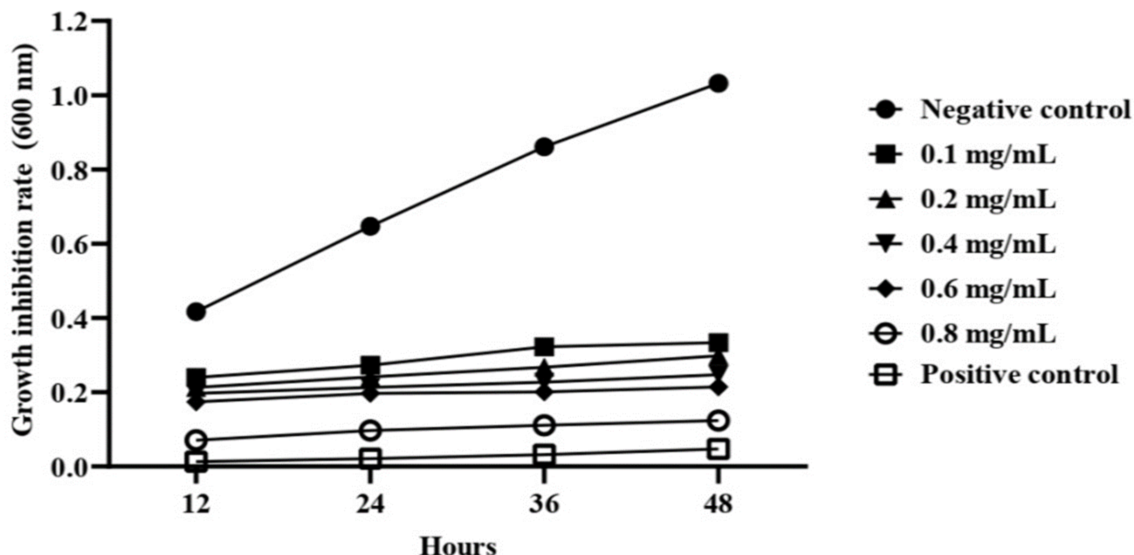


Figure 3: C^{13} NMR.

Table 1: ABTS and DPPH antioxidant assay of ethanol extract and ascorbic acid as control.

Conc. µg/mL	5	10	20	40	80	160	320
Ascorbic acid[1]	6.214977	41.21243	63.52522	78.1457	83.95313	91.54356	94.6001
Ethanol Extract[1]	8.762099	9.220581	42.68976	68.87417	85.37952	88.33418	94.9567
Ascorbic acid[2]	4.97661	61.1655	66.7801	82.1353	87.8775	90.5147	96.7673
Ethanol Extract[2]	4.04083	9.86814	16.4185	53.1689	68.6516	84.0919	88.0476

[1]ABTS method, [2]DPPH method.



Graph 1: *In vitro* study of ethanol extract at various concentrations on the growth of *Ralstonia solanacearum* with negative control.

EE possess better antioxidant activity when compared to standard ascorbic acid IC_{50} 18.27 μg/mL, Ethanol extract shows the highest antioxidant activity (15.5) by ABTS method. IC_{50} of ethanol extract 46.82 μg/mL to that of standard ascorbic acid 13.38 μg/mL by DPPH method, the difference due to specificity of compounds to the reducing compounds in the ethanol extract.

Macro-dilution

The *in vitro* study reveals rate of inhibition increases to an increase in the concentration of extract with time, hence concentration and rate of inhibition directionally proportional. 48 hr long test with 12 hr of gap with 5 different concentrations of ethanol extract, negative control with only *R. solanacearum* in liquid medium and positive control with ampicillin. The growth of bacteria in all the group predicted in the Graph 1.

In vitro study of ethanol extract at various concentrations on the growth of *Ralstonia solanacearum* with negative control. The concentration of 0.8 mg/mL of ethanol extract was found to inhibit 92% of the growth of *R. solanacearum* when compared to ampicillin (95%). 50% of growth inhibited at 0.1 mg/mL of ethanol extract at 12 hr of incubation calculation of IC_{50} was 0.11 mg/mL with the formula Y=MX+C and proved the same. In other words, the best results were obtained using 0.8 mg/mL (available upon request).

Pot culture

The symptoms of disease decreased on the tomato plant treated with ethanol extract compared to the plant treated only with water (control). The ethanol extract concentration fixed based on the *in vitro* study calculation of IC_{50} and its value 0.11 mg/mL, hence 0.05, 0.1 and 0.2 mg/mL of extract were tested on tomatoes. Plants inoculated with *R. solanacearum* (BW-1×108 CFU/mL)) were divided into two groups, plants treated with the extract (test group) and the other group was treated with only water (control). The test group further divided into 3 sub-groups (0.05, 0.1, 0.2 mg/mL) based on the concentration of ethanol extract it received. The plants treated with extract showed a significant reduction of disease compared with plants treated with water (control). The 45 days long observation reveals a 100% delay in the initial stage of infection in tomatoes in other words there is no symptoms till 25th day in the group treated with extract, then slowly symptoms started to appear after 25 days in treated group. The control group already started showing symptoms of disease. The severity of disease in later stage remarkably reduced in extract treated tomatoes received 0.1% ethanol extract significant reduction of disease (86, 74, 62, 61% of reduction on 30, 35, 40, 45 days after inoculation) ANOVA test confirms significant *p*-value less than 0.05. The tomato plants received 0.05 mg/mL of extract 64, 52, 38 and 50% rate of decrease in severity, the plants received 0.2 mg/mL of extract 57, 42, 28 and 39% rate of reduction of disease. This experiment was only confined to the greenhouse as *R. solanacearum* quarantine plant bacterial pathogen.

It was found the absence of any toxic compounds in the ethanol extract spray at specific concentration as it promotes plant growth. A tomato plants that received extract showed a more positive vegetative reaction than the control plant. The study made with 4 groups of tomato plants, the group received 0.05 mg/mL of ethanol extract, a group received 0.1mg/mL, another group received 0.2 mg/mL of ethanol extract and the last group received only water. 14% increase in vegetation, 70% increase in flowering, 103% of increase in fruit setting of Tomatoes received 0.1 mg/mL extract, the plants shows 10% of increase of vegetation, 42% increase of flowering, 66% of fruit setting received 0.05 mg/mL

Table 2: Drug-likeness scores.

P. No.	Compound Name	Formula	Molecular Weight	Water Solubility	Gastro-intestinal absorption	Lipinski Rule for Drug-Likeness	Bio-availability Score
1	2-Methyl-3-prop-1-enyloxirane	$C_6H_{10}O$	98.14 g/mol	Very soluble	High	Yes; 0 violation	0.55
2	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11 g/mol	Very soluble	High	Yes; 0 violation	0.55
3	3,5-Dimethyl-1-di methylphenylsilyloxybenzene	$C_{16}H_{20}OSi$	256.41 g/mol	Moderately soluble	High	Yes; 0 violation	0.55
4	Cyclododecane	$C_{12}H_{24}$	168.32 g/mol	Soluble	Low	Yes; 1 violation: MLOGP>4.15	0.55
5	Hexose	$C_6H_{12}O_6$	180.16 g/mol	Highly soluble	Low	Yes; 0 violation	0.55
6	Myristic acid	$C_{14}H_{28}O_2$	228.37 g/mol	Moderately soluble	High	Yes; 0 violation	0.85
7	Palmitic acid	$C_{16}H_{32}O_2$	256.42 g/mol	Moderately soluble	High	Yes; 1 violation: MLOGP>4.15	0.85
8	Heptadecafluorononanoic acid, tridecyl ester	$C_{22}H_{27}F_{17}O_2$	646.42 g/mol	Insoluble	Low	No; 2 violations: MW>500, MLOGP>4.15	0.17
9	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46 g/mol	Moderately soluble	High	Yes; 1 violation: MLOGP>4.15	0.85
10	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.45 g/mol	Moderately soluble	High	Yes; 1 violation: MLOGP>4.15	0.85
11	9,12-Octadecadienoic acid	$C_{18}H_{32}O_3$	280.45 g/mol	Moderately soluble	High	Yes; 1 violation: MLOGP>4.16	0.85
12	Icos-13-enoic acid	$C_{20}H_{38}O_2$	310.51 g/mol	Poorly soluble	Low	Yes; 1 violation: MLOGP>4.15	0.85
13	Octadeca-9,17-dienal	$C_{18}H_{32}O$	264.45 g/mol	Moderately soluble	High	Yes; 1 violation: MLOGP>4.15	0.55
14	Hexadec-9-enal	$C_{16}H_{30}O$	238.41 g/mol	Moderately soluble	High	Yes; 1 violation: MLOGP>4.15	0.55
15	Zinc, bis[[5,5'-methylenebis [3,4-dihydro-4,4-dimethyl-2H-py rrol-2-onato]] (1-)-N1,N1']-, (T-4)-	It's a complex					
16	1,4-Methanonaphthalen-9-ol, 1,2,3,4-tetrahydro-, stereoisomer	$C_{11}H_{12}O$	160.21 g/mol	Soluble	High	Yes; 0 violation	0.55
17	Octadeca-9,17-dienal	$C_{18}H_{32}O$	264.45 g/mol	Moderately soluble	High	Yes; 1 violation: MLOGP>4.15	0.55
18	2-Methyl-3,13-octadecadienol	$C_{19}H_{36}O$	280.49 g/mol	Moderately soluble	High	Yes; 1 violation: MLOGP>4.15	0.55

of extract and plants received 0.2 mg/mL shows 10% of increase in vegetation, 40% of flowering and 47% of fruit setting when compared control group.

Docking

The docking study conducted to score drug likeliness for all identified phytochemicals in *T. chebula* dried fruit ethanol extract and selected based on score (Table 2). The compound number 1, 2, 3, 5, 6 and 16 were got desirable score and obeys Lipinski's rule. Only those phytochemical's affinity to citrate synthase an enzyme catalyst in citric acid cycle and Dihydrolipoamide dehydrogenase a part of energy producing complex pyruvate dehydrogenase of *R. solanacearum* measured with molecular docking to see their significant blocking the metabolism of *R. solanacearum* hence making it as a good organic alternate for controlling bacterial wilt of tomato. Even though 9-Octadecenoic acid possess antioxidant properties, anti-bacterial and medicinal value it doesn't obey Lipinski's rule (violation 1) so omitted for being drug of choice against RS. The Palmitic acid violates Lipinski's rule 1 also excluded from the candidate even though it is second most abundant compound in the extract.

Molecular docking binding energy score confined to only 6 phytochemical compounds present in ethanol extract, Peak number 3-3,5-Dimethyl-1-dimethylphenylsilyloxybenzene contain silicon even though it obey Lipinski's rule can't be dock. Remaining 5 compounds peak number 1, 2, 5, 6 and 16 respectively 2-Methyl-3-prop-1-enyloxirane, 2-Methyl-3-prop-1-enyloxirane, 5-Hydroxymethylfurfural, Hexose, Myristic acid and 1,4-Methanonaphthalen-9-ol, 1,2,3,4-tetrahydro-(stereoisomer) binds with Dihydrolipoamide dehydrogenase of Ralstonia solanacearum and affinity were found to be -4.2, -5.1, -6, -5.3 and -6.2 with type of chemical bonds between enzyme and compound. Like-wise affinity score between citrate synthase and 5 phytochemical compounds were -3.7, -4.9, -6.4, -4.3 and -6.1.

CONCLUSION

R. solanacearum[30-33] is a quarantine organism hence the work restricted to pot culture and not proceeded with field study. Where the bioactivity of ethanol extract was tested on the tomato plants and their interaction with plant and its pathogen were made by growth, vegetation and the disease severity were compared and these parameters helped us to conclude the ethanol extract in tomato plants protects against bacterial wilt caused by *R. solanacearum*. 9-Octadecenoic acid major constituent of ethanol extract violating Lipinski rule of 5, but 1,4-Methanonaphthalen-9-ol, 1,2,3,4-tetrahydro-, stereoisomer, Hexose, 5-Hydroxymethylfurfural, Myristic acid and 2-Methyl-3-prop-1-enyloxirane. These phytochemicals control the bacterial wilt by inhibiting citrate synthase and Dihydrolipoamide dehydrogenase of *R. solanacearum* ceases the growth and reduce the incident of disease in tomatoes, can be used as a seed treatment and also as foliar spray even though single spray enough to minimise infection but a second spray after 10 days of 1st application gave more protection in the tested tomato plants against bacterial wilt also enhance the vegetation on around 25th day compared to control (29th day).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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